*DOI:*
10.1016/j.jid.2016.08.026

*Document Version*
Peer reviewed version

# Accepted Manuscript


Letter in Response to “Stabilization of β-catenin does not increase dermal papilla cell number in the hair follicle”

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PII: S0022-202X(16)32370-3

DOI: 10.1016/j.jid.2016.08.026

Reference: JID 517

To appear in: *The Journal of Investigative Dermatology*

Received Date: 11 August 2016

Accepted Date: 16 August 2016

Please cite this article as: Kaushal G, Rognoni E, Lichtenberger BM, Driskell RR, Kretzschmar K, Hoste E, Watt FM, Letter in Response to “Stabilization of β-catenin does not increase dermal papilla cell number in the hair follicle”, *The Journal of Investigative Dermatology* (2016), doi: 10.1016/j.jid.2016.08.026.

Dr Barbara Gilchrest
Editor in Chief
Journal of Investigative Dermatology

11th August 2016

Dear Dr Gilchrest,

**JID-2016-0673 - Stabilization of β-catenin does not increase dermal papilla cell number in the hair follicle**

Thank you for your e-mail of 4th August, providing us with the opportunity to respond to the conclusions of this paper. First of all we are grateful to Dr Morgan and his colleagues for engaging in a dialogue over our own paper (Kaushal et al., 2015), which began when he was one of the reviewers. We believe that open discussion about conflicting observations is important in moving forward research – far too often results that are at odds with one another are simply ignored.

There are certainly aspects of the Chi et al study that are superior to ours. In particular, we agree that there are advantages of studying the DP in unpigmented skin and we note that the quantitation by Chi et al is based on a larger number of hair follicles, and mice, than in our study.

Chi et al suggest that our results are due to misidentification of hair follicle types. We agree that DP size alone cannot be used to identify hair follicle type. We analysed skin of P65 mice in which the HF cycle was asynchronous, so that we could report on anagen (stage IV; early anagen of the second hair follicle cycle, according to the classification of Muller-Rover et al., 2001) and telogen follicles from the same mice. We believe that Chi et al are looking at a later anagen stage. Given the low frequency of non-zigzag hairs in our samples it is unlikely that they would skew our data.

The tissue in the Kaushal et al paper was not "flash frozen" but fixed with 4% paraformaldehyde before being cryopreserved in OCT medium. Tissue preservation under these conditions is excellent (Driskell et al., 2012 & 2013). The movies in the Supplemental Material of Kaushal et al show that the 60um thick horizontal wholemounts are sufficient to capture all of the cells within a DP.

Several differences in the experimental approaches could contribute to the different results:

1. Chi et al use Cre, not CreER, for genetic labelling and thus do not obtain temporal control of Cre activity. Further no control for efficient recombination / □ catenin stabilisation using this Cre line is provided in the current or previous (Enshell-Seijffers et al., 2010) study.

2. In the 2010 Dev Cell paper by Enshell-Seijffers et al., the authors describe Corin-Cre activity as being first detected at P3 in some DP cells; however, Corin is not homogenously expressed in all DP cells until P7. In contrast, Kaushal et al treated with Tamoxifen to induce

CreER at P1 and P2, before Corin-Cre is active. The use of different promoters to drive Cre expression and the activation of Cre at different times prevents direct comparison of the results.

3. Having worked with a number of different reporter lines, in our opinion the Rosa26-tdTomato reporter mouse line of Kaushal et al is superior to the Rosa26-YFP reporter used by Chi et al, both in terms of recombination sensitivity and fluorophore expression level.

Finally, two recent papers by Zhou et al support the conclusions of Kaushal et al. These authors found that 'expression of ΔN-β-catenin in CD133+ DP cells leads to increased DP cell proliferation' and 'in line with this finding, the number of DP cells was increased in mutant hair follicles... Analysis of skin histology showed that the mean size of DPs in mutant CD133-CreERT2; Rosa-rtTA; tetO-Ctnnb1ΔN hair follicles … was increased compared with controls during early anagen stages..' (Zhou et al., 2016a). These authors have further reported that expression of a stabilized form of β-catenin promotes clonal growth of CD133-positive (CD133+) DP cells in an in vitro three-dimensional hydrogel culture and on transplantation promoted in vivo hair growth in reconstituted skin compared to control cells (Zhou et al., 2016b).

In closing, we wish to thank you once again for the opportunity to discuss our results and look forward to further important contributions from the Morgan lab on the regulation and function of the dermal papilla.

Yours sincerely,

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